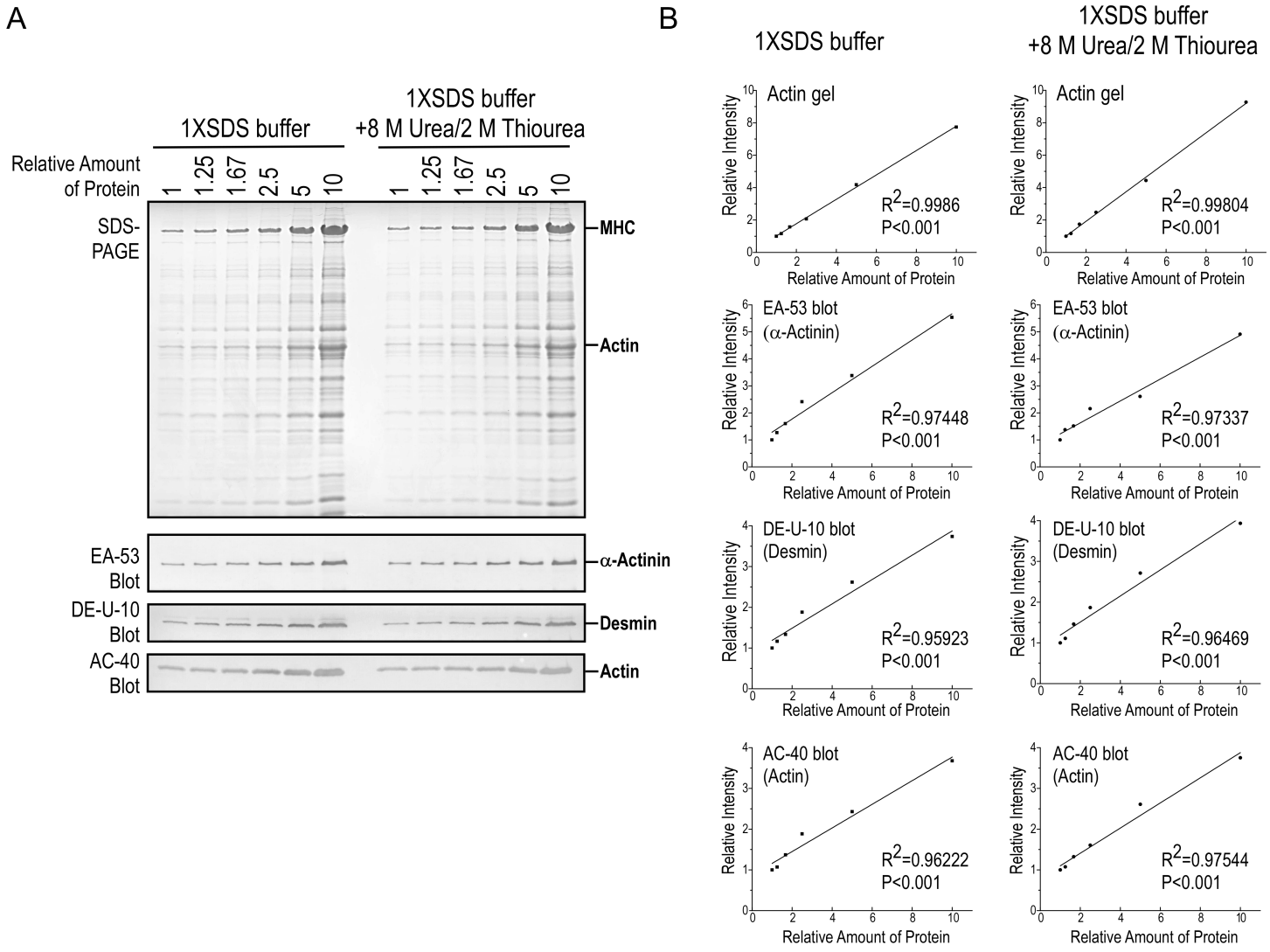
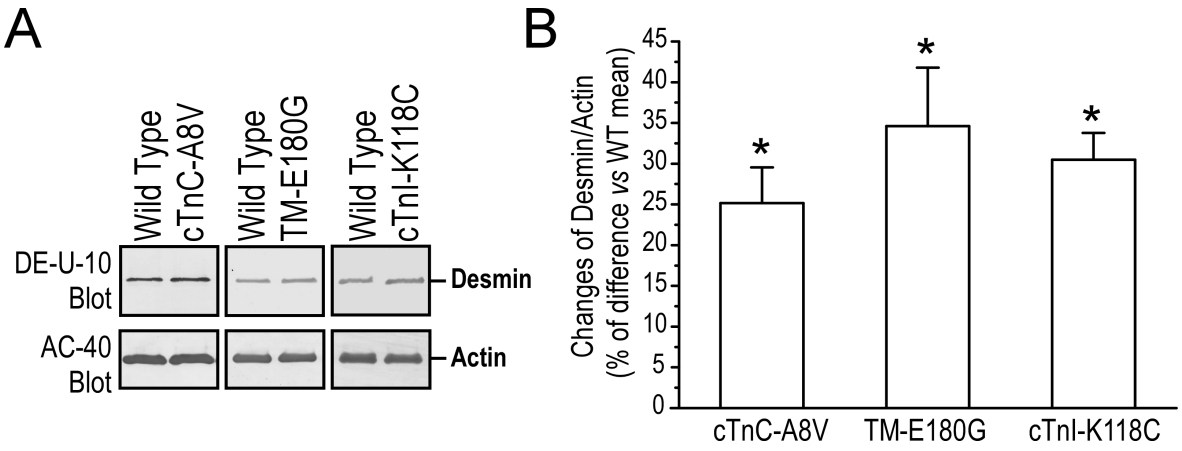
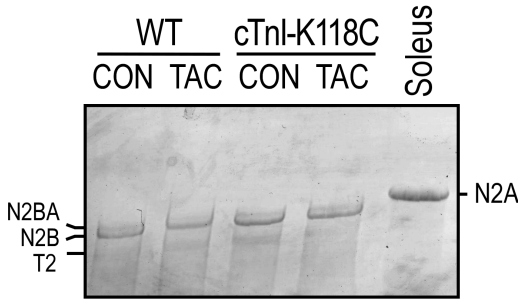


Supplement Figure 1. Reliable Western blot quantification of desmin and α-actinin. A. Representative SDS-PAGE gel and Western blots comparing protein extraction using sample buffers containing 2% SDS with or without 8 M urea and 2 M thiourea. With serial dilutions of protein extracts from adult mouse left ventricular muscle, the results showed the two extraction buffers obtained similar intensities of protein bands in Coomassie blue-stained SDS-gel and desmin, α-actinin and actin bands in Western blots using anti-desmin mAb DE-U-10, anti-α-actinin mAb EA-53 and anti-actin mAb AC-40. B. Densitometry quantifications. Excellent positive linear correlations to the protein input with a wide range were demonstrated for actin in SDS-gel and EA-53, DE-U-10 and AC-40 mAb blots. The data confirmed the reliability of quantifying desmin and α-actinin in mouse cardiac muscle using Western blot densitometry normalized to the actin bands in Coomassie blue gel or Western blot. The data shown are from representative experiments and each experiment was repeated three times with left ventricles from different mice.



Supplement Figure 2. Confirmation of increased desmin in diastolic heart failure models by normalization to Western blot of actin. A. Representative Western blots for desmin and actin using mAbs DE-U-10 and AC-40, respectively. B. Densitometry analysis normalized to actin showed that desmin was significantly increased in cTnC-A8V (25.17±4.38), TM-E180G (34.61±7.17) and cTnI-K118C (30.50±3.27) hearts as compared with wild type controls. The data are shown as means ± SEM, n=4 mice for Wild type, n=10 mice for TM-E180G, n=5 mice for cTnC-A8V and n=5 mice for cTnI-K118C groups.



Supplement Figure 3. No difference in titin splice forms in wild type and cTnI-K118C mouse left ventricular muscle before and after 2-week TAC. Vertical agarose gel electrophoresis was performed using the method modified from previous publications [1] [2]. Representative agarose gel showed similar titin bands in wild type and cTnI-K118C mice before and after 2-week TAC. The results showed the expected N2BA and N2B splice forms in adult mouse cardiac muscle samples and the soleus muscle showed only N2A form of titin. T2 is a breakdown product of titin normally seen in muscle protein extracts.

[1] Warren CM, Krzesinski PR, Greaser ML. Vertical agarose gel electrophoresis and electroblotting of high-molecular-weight proteins. Electrophoresis. 2003;24:1695-702.
[2] Reed PW, Densmore A, Bloch RJ. Optimization of large gel 2D electrophoresis for proteomic studies of skeletal muscle. Electrophoresis. 2012;33:1263-70.